

5 What we claim is:

1. An oligonucleotide for preferentially killing cancerous cells over non-cancerous cells comprising at least two CpG moieties and a prodrug for an antimetabolite covalently linked to the oligonucleotide.

10 2. The oligonucleotide of claim 1, wherein the antimetabolite is selected from the group consisting of 2'-deoxy-3'-thiacytidine, 3'-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 2',3'-didehydro-3'-deoxythymidine, 2',3'-dideoxyinosine, 5-fluoro-2'-deoxy uridine, 2-fluoro-9-b-D-arabinofuranosyladenine, 1-B-D-arabinofuranosylcytosine, 5-azacytidine, 5-aza-2'-deoxycytidine, 6-mercaptapurineriboside, 2-chlorodeoxyadenosine, and pentostatin.

3. The oligonucleotide of claim 1, wherein the prodrug is a prodrug for the antimetabolite 2'-deoxy, 2',2'-difluorocytidine.

20 4. The oligonucleotide of claim 1, wherein two of the at least two CpG moieties are separated by a number of nucleotides selected from the numbers 2, 5, and 9.

5. The oligonucleotide of claim 1, wherein said prodrug is 5' to the at least two CpG moieties.

25

6. The oligonucleotide of claim 1, wherein said prodrug is 3' to the at least two CpG moieties.

7. The oligonucleotide of claim 1, wherein said prodrug is 3' to at least one CpG moiety and 5' to at least a second CpG moiety.

30

8. The oligonucleotide of claim 1, wherein said prodrug is linked to the oligonucleotide by a 3'-3' linkage.

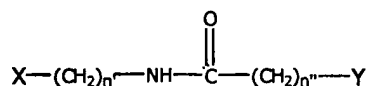
9. The oligonucleotide of claim 1, wherein said prodrug is linked to the oligonucleotide by a 5'-5' linkage.

10. The oligonucleotide of claim 1, wherein said prodrug is linked to the oligonucleotide by a 3'-5' linkage.

11. The oligonucleotide of claim 1, wherein said prodrug is covalently linked to the oligonucleotide by a 5'-3' linkage.

12. The oligonucleotide of claim 1, wherein said prodrug is at a position that is selected from 10 nucleotides upstream from one of the at least two CpG moieties, 9 nucleotides upstream from the CpG moiety, 8 nucleotides upstream from the CpG moiety, 7 nucleotides upstream from the CpG moiety, 6 nucleotides upstream from the CpG moiety, 5 nucleotides upstream from the CpG moiety, 4 nucleotides upstream from the CpG moiety, 3 nucleotides upstream from the CpG moiety, 2 nucleotides upstream from the CpG moiety, 1 nucleotides upstream from the CpG moiety, 10 nucleotides downstream from a CpG moiety, 9 nucleotides downstream from the CpG moiety, 8 nucleotides downstream from the CpG moiety, 7 nucleotides downstream from the CpG moiety, 6 nucleotides downstream from the CpG moiety, 5 nucleotides downstream from the CpG moiety, 4 nucleotides downstream from the CpG moiety, 3 nucleotides downstream from the CpG moiety, 2 nucleotides downstream from the CpG moiety, and 1 nucleotides downstream from the CpG moiety.

13. The oligonucleotide of claim 1, wherein the prodrug is covalently linked to the oligonucleotide by a linker having the formula.



wherein x and y are independently selected from  $\begin{array}{c} O \\ || \\ -P- \\ | \\ R \end{array}$ ,  $\begin{array}{c} O \\ || \\ C=O \\ | \\ O \end{array}$ , and  $CH_2$

and R is selected from H, S, a C<sub>1</sub>-C<sub>6</sub> alkyl, a C<sub>1</sub>-C<sub>6</sub> alkoxy, and NH.

5

14. The oligonucleotide of claim 1, wherein the oligonucleotide comprises at least one nucleotide having a ribose sugar moiety.

10

15. The oligonucleotide of claim 1, wherein the oligonucleotide comprises at least one nucleotide having a 2'-deoxyribose sugar moiety.

15

16. The oligonucleotide of claim 1, wherein the oligonucleotide comprises at least one 2'-halogen nucleotide.

20

17. The oligonucleotide of claim 1, wherein the oligonucleotide comprises at least one 2'-N-alkyl nucleotide wherein the alkyl has between about 1 and about 6 carbon atoms.

18. The oligonucleotide of claim 1, wherein the oligonucleotide comprises at least one 2'-O-alkyl nucleotide, one 2'-N-Alkyl nucleotide, or one 2'-O-halogen nucleotide, wherein the alkyl has between about 1 and about 6 carbon atoms

25

19. The oligonucleotide of claim 19, wherein the alkyl is methyl.

30

20. The oligonucleotide of claim 1, wherein the oligonucleotide comprises a plurality of nucleotides connected by covalent internucleoside linkages, wherein each of the linkages are selected from the group consisting of a phosphodiester linkage, a C1-C6 alkoxy phosphotriester linkage, a phosphorothioate linkage and a phosphoramidate linkage.

21. A pharmaceutical composition comprising a therapeutically effective amount of the oligonucleotide of any of claims 1-20.

35

5            22.     The oligonucleotide of claim 21 wherein said pharmaceutically acceptable carrier is lipofectin.

             23.     An oligonucleotide for preferentially killing cancerous cells over non-cancerous cells comprising a motif represented by the formula: 5'PGXCG3' wherein P is  
10   a prodrug for an antimetabolite and X represents between 0 and 50 nucleotides.

             24.     The oligonucleotide of claim 23, wherein the antimetabolite is 2'-deoxy, 2',2'-difluorocytidine.

15           25.     The oligonucleotide of claim 23, wherein the metabolite is selected from the group consisting of 2'-deoxy-3'-thiacytidine, 3'-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 2',3'-didehydro-3'-deoxythymidine, 2',3'-dideoxyinosine, 5-fluoro-2'-deoxy uridine, 2-fluoro-9-b-D-arabinofuranosyladenine, 1-B-D-arabinofuranosylcytosine, 5-azacytidine, 5-aza-2'-deoxycytidine, 6-mercaptopurineriboside, 2-  
20   chlorodeoxyadenosine, and pentostatin.

             26.     The oligonucleotide of claim of 23, where X is selected from the group consisting of 2, 5, and 9.

25           27.     The oligonucleotide of claim 23, wherein the oligonucleotide comprises multiple nucleotides and the prodrug is covalently linked to one of the nucleotides by a 3'-3' linkage.

             28.     The oligonucleotide of claim 23, wherein the oligonucleotide comprises  
30   multiple nucleotides and the prodrug is covalently linked to one of the nucleotides by a 5'-5' linkage.

             29.     The oligonucleotide of claim 23, wherein the oligonucleotide comprises multiple nucleotides and the prodrug is covalently linked to one of the nucleotides by a  
35   3'-5' linkage.

29. The oligonucleotide of claim 23, wherein the oligonucleotide comprises multiple nucleotides and the prodrug is covalently linked to one of the nucleotides by a 3'-5' linkage.

30. The oligonucleotide of claim 23, wherein the oligonucleotide comprises multiple nucleotides and the prodrug is covalently linked to one of the nucleotides by a 5'-3' linkage.

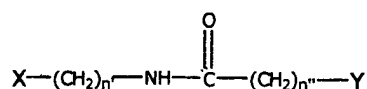
31. The oligonucleotide of claim 23, wherein the oligonucleotide comprises at least one nucleotide having a ribose sugar moiety.

32. The oligonucleotide of claim 23, wherein the oligonucleotide comprises at least one nucleotide having a 2'-deoxyribose sugar moiety.

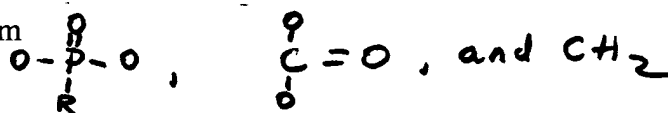
33. The oligonucleotide of claim 23, wherein the oligonucleotide comprises at least one 2'-O-Alkyl nucleotide, 2'-N-Alkyl nucleotide, or 2'-O-halogen nucleotide, wherein the alkyl has between about 1 and about 6 carbon atoms.

34. The oligonucleotide of claim 23, wherein the oligonucleotide comprises a plurality of nucleotides connected by covalent internucleoside linkages, wherein the linkages are selected from the group consisting of phosphodiester linkage, a C1-C6 alkoxy phosphotriester linkage, a phosphorothioate linkage and a phosphoramidate linkage.

35. The oligonucleotide of claim 23, wherein the oligonucleotide comprises multiple nucleotides and the prodrug is attached to at least one of the multiple nucleotides by a linker having the formula



wherein x and y are independently selected from



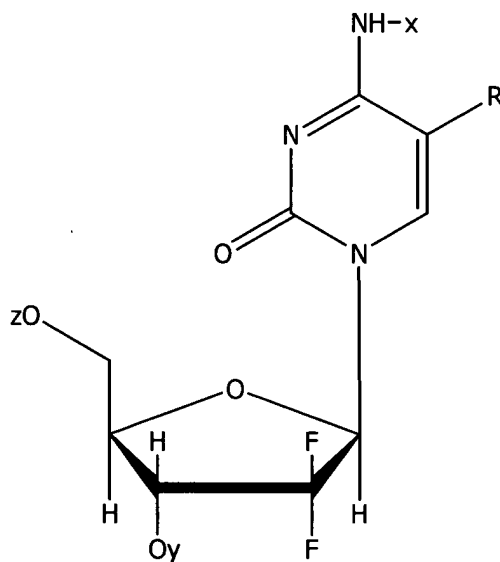
and R is selected from H, S, a C<sub>1</sub>-C<sub>6</sub> alkyl, a C<sub>1</sub>-C<sub>6</sub> alkoxy, and NH.

5            36.    A pharmaceutical composition comprising a therapeutically effective amount of the oligonucleotide of any of claims 23-35.

             37.    The oligonucleotide of claim 36 wherein said pharmaceutically acceptable carrier is lipofectin.

10

             38.    A compound having purity in excess of 98% by HPLC, having the formula:



15

             wherein R is selected from the group consisting of H, a C1-C6 alkyl, a halogen, a C2-  
20    C6 alkenyl, and a C2-C6 alkynyl;

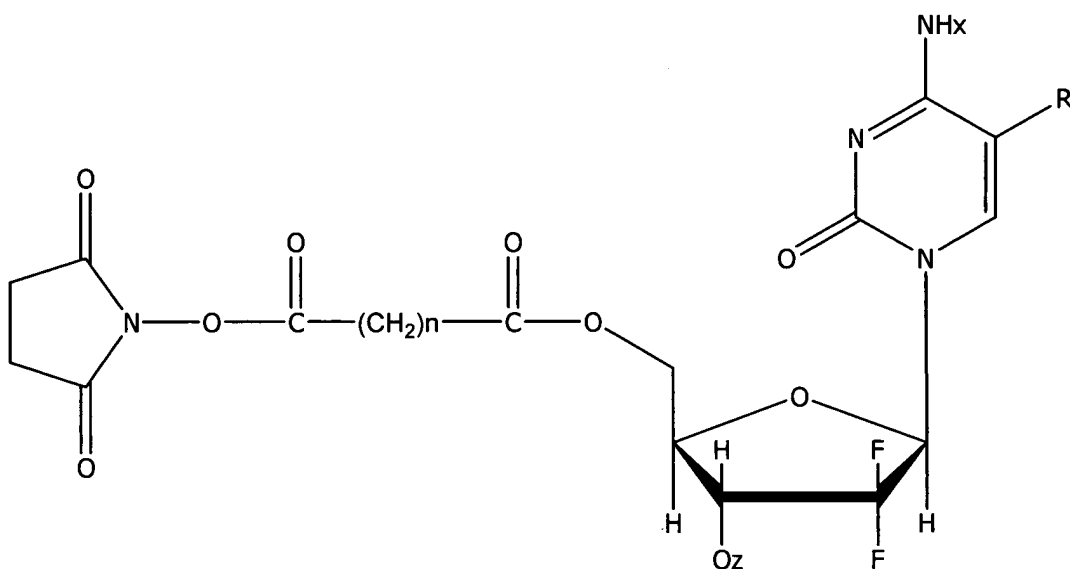
             x is an amine-protecting group that is stable in oligonucleotide synthesis conditions;  
             and

- 5 y, and z are each selected from H, a hydroxyl-protecting group that is stable in oligonucleotide synthesis conditions and a group that can be attached to a solid support.

39. The compound of claim 23, wherein the group that is attachable to a solid  
10 support has the formula  $O-C(=O)-M-C(=O)-NH$ -Spacer, where M is selected from the group consisting of succinyl, oxalyl, and hydroquinolynyl, and wherein the Spacer is selected from the

40. group consisting of a C1-C6 alkyl, ethyloxyglycol, and a combination of alkyl and ethyleneglycoxy.

15 41. A compound having the formula:



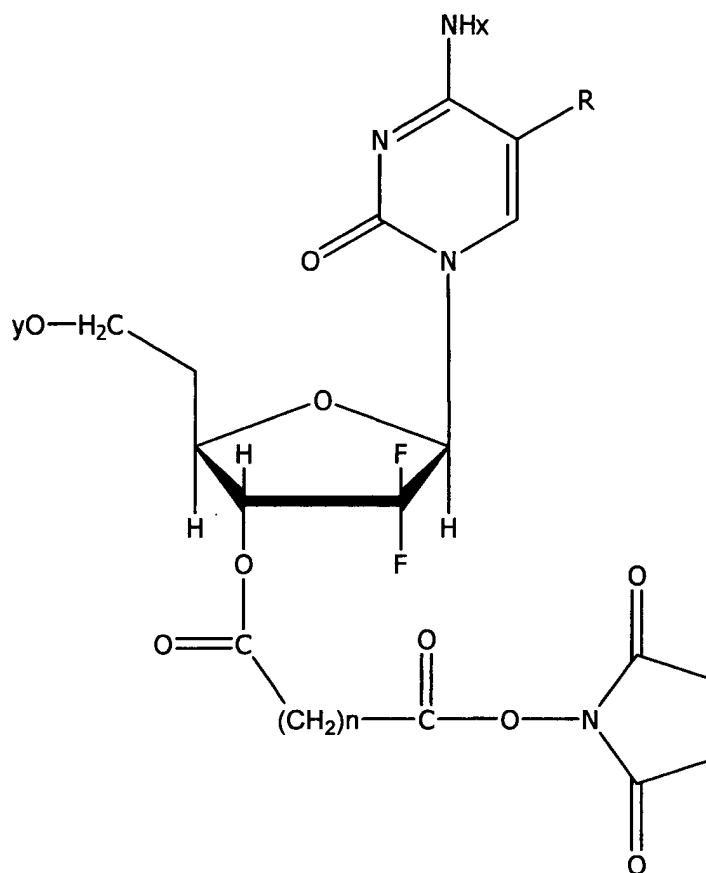
20 wherein R is selected from the group consisting of H, a C1-C6 alkyl, a halogen, a C2-C6 alkenyl, and a C2-C6 alkynyl;

25 x is an amine-protecting group that is stable in oligonucleotide synthesis conditions;

5 z is a hydroxyl-protecting group that is stable in oligonucleotide synthesis conditions; and

n is 2-20.

42. A compound of the formula:



10

wherein R is selected from the group consisting of H, a C1-C6 alkyl, a halogen, a C2-C6 alkenyl, and a C2-C6 alkynyl;

15 x is an amine-protecting group that is stable in oligonucleotide synthesis conditions;

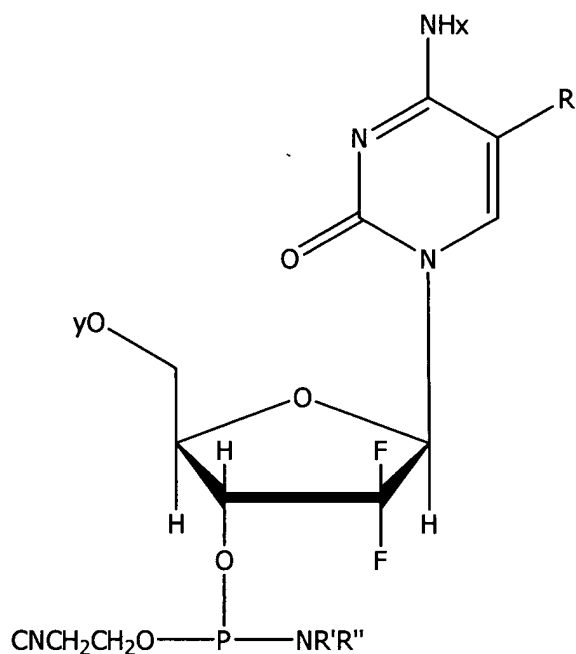
z is a hydroxyl-protecting group that is stable in oligonucleotide synthesis conditions; and

n is 2-20.

20



- 5            43.     A compound having a purity in excess of 97% by HPLC, as shown by the formula:



10

wherein y is a hydroxyl-protecting group that is stable in oligonucleotide synthesis conditions;

x is an amine-protecting group that is stable in oligonucleotide synthesis conditions;

15

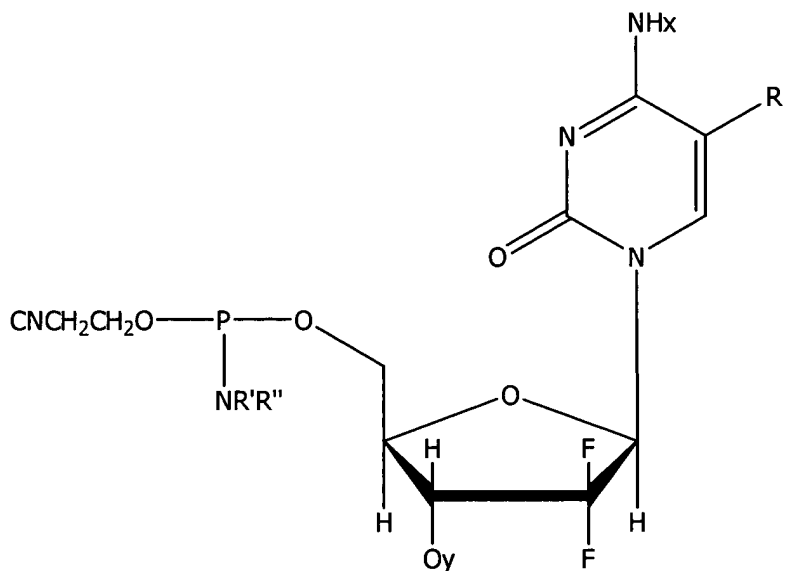
R is selected from the group consisting of H, a C1-C6 alkyl, a halogen, a C2-C6 alkenyl, and a C2-C6 alkynyl; and

R' and R'' are independently selected from the group consisting of a C1-C6 alkyl and a C2-C6 cycloalkyl.

20

44.     A compound having purity in excess of 97 % by HPLC, and having the formula:

5



wherein y is a hydroxyl-protecting group that is stable in oligonucleotide synthesis conditions;

10

x is an amine-protecting group that is stable in oligonucleotide synthesis conditions;

R is selected from the group consisting of H, a C1-C6 alkyl, a halogen, a C2-C6 alkenyl, and a C2-C6 alkynyl; and

15

R' and R'' are independently selected from the group consisting of a C1-C6 alkyl and a C2-C6 cycloalkyl.

20